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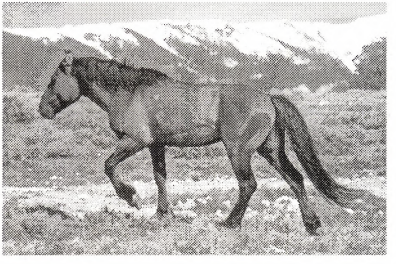
Field Trial Plan
Wild Horse Fertility Control

U.S. Department of Interior, Bureau of Land Management and Biological Resources Discipline of the U.S. Geological Survey,
Natural Resource Ecology Laboratory of Colorado State University
and
Science and Conservation Center of Billings, Montana

Treatment of Wild Horse Mares with the Immunocontraceptive Porcine Zonae Pellucida Vaccine; Effects on Populations and Behavior



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EXECUTIVE SUMMARY

The Bureau of Land Management (BLM) has an immediate need for a safe, effective contraceptive agent to assist in the management of the large number of wild horses on western rangelands. The BLM is directed to manage horse numbers at levels compatible with the range under the Wild Free-Roaming Horse and Burro Act of 1971. The BLM and the US Geological Survey-Biological Resources Division seek to test the immunocontraceptive agent *Porcine Zonae Pellucida* (PZP) in field trials with three small populations and 8-20 large populations of free-roaming western wild horses. Extensive research has already been conducted on the safety, efficacy, and duration of PZP applications in both domestic and feral horses on eastern barrier islands and in a few select trials with wild horses in Nevada managed by the BLM. However, significant questions remain concerning the effects of PZP application at the population level as well as the effects on behavior, social structure, and harem dynamics of free-ranging animals. These questions are best answered with field trials on wild horse herds under a research protocol.

This plan details protocols for injections, experimental design, and research methods that will be employed to evaluate effects of PZP on free-ranging animals. The research will focus on the effects of immunocontraceptive treatment on seasonality of foaling, any possible compensatory reproduction of mares post-treatment, duration of estrus cycles, population growth rates, and harem behavior. Following either three or four years of treatments (22 months in the large populations), the subject mares will remain in the wild herds, and they will not be treated again for the remainder of their lives. The behavior and fertility of the treated mares will be studied both during the treatment phase, and for a minimum of two years post-treatment to assure that a return to normal fertility occurs.

Permission to conduct research using PZP is covered under an Investigational New Animal Drug Exemption (INAD #8857) filed with the Food and Drug Administration (FDA) by the Humane Society of the United States (HSUS). All herd management areas included in this field trial plan must provide approved gather plans and Environmental Assessments (EAs) detailing the contraception research before the research can be initiated in any specific area.

The U.S. Geological Survey, Biological Resources Division (USGS-BRD), will provide the funding for the purchase and injections of the PZP, provide research oversight, hire biological technicians, and pay for aerial surveys and monitorings of the study herd areas. USGS-BRD will assure that the appropriate Animal Care and Use Committee (ACUC) provides input into and approves the animal handlings. The BLM, through the BLM WH&B research coordinator and Reno-based National Policy Office (NPO) field office, will provide coordination with herd area managers; approvals from, and any necessary logistic support at, the BLM field office level; assurances that the necessary NEPA and any other approval documents are in place; coordination with the media and the public; and will support capture and handling of the animals. Field trials will be initiated in July 2002.

The intent of this research field trial effort is to answer those remaining questions and concerns about fertility control using PZP that are best answered on free-ranging populations in the wild. The ultimate goal is to provide the BLM with the protocols and information necessary to begin to use fertility control to assist in population regulation of wild horse herds on a broader scale. Fertility control is intended to assist the conventional capture, removal, and adoption

process, and to greatly reduce the adoption costs and numbers of animals handled. Fertility control is not intended to totally replace the removal and adoption process.

I. THE MANAGEMENT AND RESEARCH CHALLENGE

The Wild Free-Roaming Horse and Burro Act of 1971 (The Act), as amended, provides for the protection and management of wild horses and burros to ensure a thriving, natural ecological balance and multiple-use relationship on the range. As directed in The Act, the Bureau of Land Management (BLM) is responsible for managing healthy, viable wild horse and burro populations within herd management areas (HMAs) at appropriate management levels and through appropriate placement of excess animals.

When the Wild Horse and Burro Act of 1971 was passed, a roughly estimated 17,000[1] wild horses lived on public lands. By 1980, that number had increased to between 65,000 and 85,000 animals. Natural predators of horses (wolves, bears, and mountain lions) are currently not present, or are found in low numbers, on all but a few of the wild horse ranges in the western United States. Hence, survival rates of wild horses have been very high (adult annual survival rates exceeding 95%) in most herds, and many horse herds grow at sustained high rates of 15-22% per year.

Although wild horses occur in 10 states, the vast majority of animals are located in Nevada and Wyoming. The BLM has tentatively determined the most appropriate management level (AML) for most managed rangelands, although AMLs have not officially been set for all herd management areas. Current numbers of wild horses are still substantially in excess of the AML goals set by the BLM. A Presidential budget initiative in 2000 provided BLM's Wild Horse and Burro Program the opportunity to increase gather efforts over the next five years, in efforts to reduce wild horse numbers to AML goals.

A major challenge for the BLM is placing the large numbers of excess horses removed from the range each year. Currently, placement of excess animals is through BLM's Adopt-A-Horse program. However, not all wild horses and burros can successfully be placed in adopted homes and many unadoptable animals are kept for years in captivity in long-term pasture holding facilities.

There is an immediate need for less costly and more efficient and humane management of wild horses on public lands. Contraception has long been recognized as a humane alternative to limit the growth of wild horse herds, and it can reduce the number of animals handled and handling costs. Contraception may also provide less disruption to the herd's gene pool. The gather and removal program regularly and permanently manipulates the gene pool, while reversible contraception would leave more genetic material in the herd for natural selection. Research into the use of contraceptives has been ongoing since the 1970s, but within the last two decades more emphasis has been placed on immunocontraception. The leading immunocontraceptive vaccine contains the antigen Porcine Zonae Pellucida (PZP). The agent

[1] Systematic aerial surveys were not conducted during this period, and this number must be viewed as a rough approximation.

includes an adjuvant, which helps the immune system recognize the antigen. Recent efforts have yielded, in addition to the conventional PZP, a time-release PZP; a field-deliverable, one-injection vaccine containing polymer pellets that release PZP, providing up to two-years of efficacy (actually 22 months). Over the last 14 years, PZP has been successfully applied to control fertility and limit the size of several small populations of wild horses on eastern barrier islands (Cape Lookout National Seashore, Shackleford Banks; Carrot Island, Rachel Carson National Estuarine Reserve; and Little Cumberland Island, a private island). Over the last decade, both conventional and time-release PZP have also been tested in limited trials.

Fertility control is not intended to completely replace the management need for removals and adoptions. However, fertility control can reduce the total number of animals that need to be adopted, handled, or held in captive situations, as well as the frequency, size, and cost of gathers.

II. BACKGROUND

PZP appears to meet many of the stated BLM requirements for an ideal contraceptive agent including criteria for safety and efficacy (Appendix 1-4). There is currently more knowledge about the effects of PZP on horses and there has been more management of wild horse herds (mostly on eastern barrier islands) using PZP than any other contraceptive agent. The vaccine has been relatively inexpensive ($25 per dose; except recent costs for time-release doses are $200), can be remotely administered in the field, and requires a single annual booster dose to confer infertility for one breeding season. PZP contraception is reversible following the three to four years of treatment proposed. PZP treatments may not be reversible following longer time periods of five to seven years of continuous treatment. PZP has demonstrated no impact on already developing foals in treated mares and has no known ill effects on ovarian function for treatments limited to 2-4 years. Progeny of treated mares, following cessation of treatments, also reproduce and behave normally. Treated mares can return to normal fertility following cessation of treatments (up to 4 years), and treatments may, in fact, improve body condition and increase longevity of mares.

In spite of the generally favorable initial experience with PZP, field applications to date have been very limited. PZP has been used for long-term population management in only one herd of feral horses (Assateague Island, Maryland). Field applications with free-ranging BLM wild horses in Nevada have been limited to trials with a small number of mares and primarily remote (aerial) monitoring of foaling by treated mares. While no noteworthy problems have been observed, the BLM, Animal and Plant Health Inspection Service (APHIS), and USGS-BRD seek to further test PZP under a tight research protocol in a series of field trials. A number of unanswered questions concerning the effects of PZP on population viability, harem structure, and estrus cycling must be addressed before PZP can be recommended for large scale management applications. Additionally, there are some concerns that the adjuvant currently used, Freund's Complete Adjuvant (FCA), can cause some health problems. Ovarian function needs to be assessed for a large sample of mares treated for four years.

The current PZP product meets many, but not all, of BLM's needs for an agent for broadscale use. Conventional PZP and Time-Release PZP can be used to manage BLM's smaller wild horse herds. However, a longer-lasting single shot agent that would provide either three or four years of continuous contraception is needed for BLM's larger herds where gathers

and removals occur every four years. If a longer-lasting agent could be found, treated mares would only need to be captured and treated once in their lifetime, thus saving considerable cost and handling stress to the animals. PZP might be modified to meet the longer-lasting needs, or perhaps another longer-lasting agent will meet these needs.

A parallel research effort to produce a three to four year agent and to test alternative adjuvants[2] is proposed under the larger research umbrella outlined in the strategic research plan. That parallel effort is proposed to focus on: (a) refinement of controlled-release technology to yield a PZP vaccine of three to five years duration; (b) development of a "dry," completely pelletized PZP vaccine, which permits low-cost vaccine shipping, long-term vaccine storage, and simple vaccine preparation/delivery; and (c) continued recruitment and testing of replacement adjuvants to Freund's Complete for use in primer and controlled-release pellet booster preparations. This is important because, despite current permission to use FCA in the PZP vaccine under the Investigational New Animal Drug Exemption (INAD) research plan, the long-term goal of the BLM and USGS-BRD is to replace FCA with another adjuvant that will be more acceptable to the Food and Drug Administration (FDA).

Significant questions remain concerning the broad-scale use of PZP in free-ranging herds of wild horses in the western states that are now best answered with field trials with free-ranging wild horses. There is concern over the adjuvant currently used in PZP mixtures. Many believe the adjuvant currently used, FCA, can cause some health problems. The research team is seeking an alternative adjuvant to FCA. FCA causes a false positive tuberculosis test, and can cause granulomas at the site of injection in a percentage of treated mares. These granulomas are generally small and shrink over time, providing the injection site was in the buttock area. Only certified/trained people should handle agents that include FCA. Zoo Montana has trained BLM specialists in the past to handle the PZP and FCA mixture. Modified Freund's Adjuvant, QS-21, and other existing adjuvants, may be potential replacements, but the efficacy and duration of these potential replacements needs to be evaluated under controlled conditions. The agencies will substitute a new adjuvant as soon as an effective replacement can be found.

The following issues need to be addressed by the BLM, USGS-BRD and APHIS before there is widespread application of PZP to all western herds, including:

- Monitoring of all treated populations is necessary to test management prescriptions under a research protocol. But most animals can only be handled during captures and are not approachable for darting. Time-release PZP will find greater application in the larger herds, but it needs to be determined if the 22-month time-release agent will exert significant controls on population growth when administered once during the normal 4-year gather cycle. The search for a longer-lasting agent should continue simultaneously as the field trials outlined here occur with conventional and the current time-release PZP.

- The effects of PZP and the adjuvant, FCA, on any clinical health problems (e.g. possible abscesses, ovarian damage), need to be observed under tightly controlled conditions.
- Compensatory reproduction may occur following contraception in free-ranging herds. The extent of this phenomenon needs to be determined and incorporated into projection

[2] Adjuvants are components of the vaccine that enhance the main product, or antigen, and consequently increase antibody formation.

models. Fertility rates of mares may be higher after cessation of contraception, since body condition of mares improves while they are contracepted. Treated mares are not subjected to the stresses of pregnancy and lactation, and mares will be in excellent body condition when the treatment effects wear off.

- Concerns exist that PZP application may influence the seasonality of birth of foals. The 22-month time-release vaccine, if applied during a summer gather, may permit mares to conceive late during the second summer following treatment and thus, give birth to late season foals the third summer following treatment. These late-born foals might survive at a lower rate as has been documented in other ungulates. Any potential influence of PZP applications on season of foaling should be sorted out from other factors that may also influence seasonality, utilizing multivariate analysis and "best model" selection employing Information Criteria. For example, density dependence and weather may also influence date of birth. Age of the stallions and age of the mare may also affect seasonality of foaling. When young adult males assume mating, breeding and births may be slightly later. Young, breeding-aged females may conceive later and give birth later than prime-aged females. Extensive pretreatment information on seasonality of births needs to be gathered during periods of varying weather conditions (severe to benign), varying densities (low to high), variable harem stallion age and composition, and variable age of mares, to sort out any other effects on seasonality of foaling from any effects due to contraception.

- Adult wild female deer treated with PZP tend to cycle later, even well into the winter. If this same pattern also occurs in PZP-treated mares, mare and stallion energetics might be negatively influenced. This is only a hypothesis. Many mares naturally do not become pregnant in wild herds, and these non-pregnant wild mares have not been observed to cycle later than August, apparently due to weather and photoperiod controls. Additionally, harem stallions steal mares and protect mares in their harems year-round. Harem holding behaviors are not limited to just estrus periods. Stallions do not extensively court or tend estrus harem mares in their harems. Thus, even if a few late estrus periods occur due to PZP treatment, there may not be any greater energetic drains on stallions or mares above normal tending and courting behaviors. Research is needed to determine whether or not late estrus occurs, and if there are any additional energetic expenditures that are biologically important.

- Contraception has been hypothesized to protect herd genetic diversity, or heterozygosity (Gross 2000), by allowing more stable population sizes and a more stable breeding group (called the effective population size, N_e), over the long term compared to traditional gather and removal programs. Contraception has the potential to disrupt natural selection, depending upon which mares are selected for treatment, although all treated mares will be allowed to return to fertility and to genetically contribute following cessation of treatments.

III. PURPOSE AND OBJECTIVES OF FERTILITY CONTROL FIELD TRIALS

The BLM requires the highest quality science to simultaneously manage for sustainable production of all components of the ecosystem, including free-roaming, healthy, and genetically viable populations of wild horses and burros. To this effort, BLM has developed a comprehensive, long-term research strategy, which identifies research needs in the areas of fertility control, herd genetics, herd health, aerial census techniques and habitat evaluations. One need identified in this strategic plan is the evaluation of PZP as a tool for herd management.

The field trials began in July 2002 and will assess vaccine effects on mare estrus, foaling, body condition, behavior, fitness and survival. The field trial plan adheres to a well-developed research protocol, and to any restrictions, requirements, advice, or suggestions placed on PZP vaccine research set by the Humane Society of the United States (HSUS), the FDA, the Animal and Plant Health Inspection Service (APHIS), the appropriate Animal Care and Use Committee, and the National Wild Horse and Burro Advisory Board.

BLM must provide, in advance of the contraceptive research, a herd management plan through the National Environmental Policy Act (NEPA) documentation (generally in the form of a gather plan and Environmental Assessment [EA]). The herd management plans must identify and justify the target numbers (AMLs) for the herds. The plans must also identify the objectives and goals for vaccine use, as well as expected protocol for application. The target number and age classes of mares, and application level must be identified. Vaccine will only be provided to HMAs where there is a stated intent to manage for healthy and viable herds.

The field trials for small populations (Individual-Based) will provide either three or four years of contraception to treated mares. Following three or four years of contraception, treated mares will be allowed to return to normal reproductive function. The purpose of the multiple years of treatment is to simulate, as nearly as possible, the effects of treatment and recovery that would occur to both the individual and the population, once a single-shot, 4-year agent becomes available. Their fecundity rates, behavior, and harem social structure will be observed for a minimum of two years post-treatment, to assure that normal fertility is resumed. The treated mares will be individually marked and/or be individually recognizable without error. These mares must be left on the range for the duration of the research, and are not to be treated again. Any study mares that are treated with both PZP and FCA may not be adopted until three years after treatment has occurred (correspondence from Humane Society of America, July 2002, concerning INAD #8857).

The field trials for large populations (Population-Based trials) will be conducted to answer primarily one question: Will the 22-month Time Release PZP exert significant effects on population growth rates, to achieve handling and economic benefits, when administered within the scope of the typical 4-year gather cycle. Treated mares will be recognized only as "treated" or "untreated" through a hip brand. Individual mares will not be required to be recognizable, although no herd manager will be discouraged from any individual recognition process (WHIMS, chip, freeze-brand, etc.) that they so desire.

The objectives of the PZP field trials are as follows:

(1) To provide the BLM with empirical data on the efficacy of PZP as a management tool to assist with population regulation.

(2) To evaluate the effects of PZP contraception on population growth rate, foaling rates, harem behaviors, estrus cycling of wild mares, and any health issues (Individual-Based).

(3) To evaluate the effects of PZP treatment on any possible compensatory reproduction or compensatory survival in the population.

(4) To evaluate the effects of PZP contraception on herd level genetic heterozygosity, fitness, and on the prevalence of matrilines or patrilines (Individual-Based).

(5) To evaluate the effects on population growth of the 22-month Time Release Agent when administered in a 4-year gather cycle (Population-Based).

IV. RESEARCH TEAM MEMBERS

The experienced team of researchers who will conduct the field trials include personnel in BLM's Wild Horse and Burro Program who have experience with herd management concerns and alternatives; nationally recognized ungulate population biologists and behavior expertise; nationally recognized expertise in equine reproduction; qualified veterinarians; ungulate control ecology expertise; and statistical and fertility control expertise.

BLM Research Coordinator:	Linda Coates-Markle, M.Sc., State of Montana Wild Horse and Burro Specialist, BiFO, BLM, and ½ time coordinator, BLM-NPO
USGS/BRD Research Principal Investigator:	Francis Singer, Ph.D., USGS-BRD, Research Ecologist and Ungulate Ecology Program Leader, Fort Collins Science Center, 2150 Centre Avenue, Bldg. C, Fort Collins, CO 80526-8118
Sole Source PZP Contractor; 1- and 2- Year Vaccine Preparation, designated darters; Training of delegated darters under their direction:	Jay F. Kirkpatrick, Ph.D., Robin Lyda and Kim Frank, The Science and Conservation Center, Zoo Montana, 2100 South Shiloh Road, Billings, MT 59106
Population Modeling; Economic Effects of Treatments	N. Thompson Hobbs, Ph D., Senior Scientist, Natural Resource Ecology Laboratory, Colorado State University, Fort Collins, CO 80526; John Bartholow and Linda Zeigenfuss, USGS-BRD[1]
Genetic Conservation:	Gus Cothran, Ph.D., Horse Genetics Lab, University of Kentucky, Lexington, KY 40506-0099 (genetic analyses; population genetics); John Gross, Ph.D., Davies Lab, Queensland, Australia (population genetics modeling)

Equine Reproductive Physiology and Equine Veterinarian:	Patrick McCue, D.V.M., Ph.D., Equestrian Reproductive Laboratory, Colorado State University, Fort Collins, CO 80525
Aerial Survey:	Bruce Lubow, Ph D., Fisheries and Wildlife Sciences, Colorado State University, Fort Collins, CO 80523 (statistical analysis, experimental design); Francis Singer, Ph D. (design, conduct aerial surveys)

ADVISORS TO THE FIELD TRIALS:

Wild Horse Health and Care Veterinarians:	Albert Kane, D.V.M., M.P.V.M., PhD. (APHIS);

V. PERMISSION AND CRITERIA FOR VACCINE USE

The Humane Society of the United States (HSUS) has made the PZP vaccine available to the BLM under the Investigational New Animal Drug Exemption (INAD #8857) filed with the FDA. As a condition of using the PZP vaccine, the HSUS expects the BLM to follow the Draft Criteria for Immunocontraceptive Use in Wild Horse Herds recommended by the Wild Horse and Burro National Advisory Board in August 1999, as well as provide details of the Fertility Control Field Trial Plan and any and all NEPA documents related to this effort to the FDA in a timely manner. The HSUS has also provided instructions for a three-year waiting period before any treated mares may be adopted.

VI. VACCINE QUALITY, DELIVERY PROTOCOL, AND ANIMAL AND HUMAN SAFETY

All PZP vaccine used on mares during the field trials will be provided by the Science and Conservation Center (SCC), Zoo Montana (or a suitable alternative source) and subjected to quality control testing (see Appendix 2). All documented aspects of PZP vaccine provision, mare selection, vaccine delivery, remote-dart recovery, record keeping, veterinary emergencies, and media relations, will be strictly adhered to by all participants in the proposed action. These protocols shall serve as the Standard Operating Procedures (SOPs) for the proposed field trial program. The SOPs take into consideration all safety concerns, individual animal health and condition (e.g., a new needle will be used for each injection), seasonal distribution of the horses, as well as local weather and environmental considerations.

Only trained and certified handlers may handle or inject the vaccine and adjuvant. In the past, training has been provided at Zoo Montana, Billings, Montana for potential BLM, USGS, APHIS, or other employees. Only Science and Conservation Center handlers, or designated certified handlers under their immediate supervision, training and direction, may handle the PZP vaccine and inject mares as detailed under this research protocol.

The BLM must provide notification, in advance, to state and area veterinarians in charge, when any unlicensed research product, either agent or adjuvant, is to be used in their area under this research protocol. The BLM will initiate these contacts by and through the National Coordinator for APHIS-BLM Wild Horse and Burro Program Partnership.

Related population modeling, detailed in the strategic research plan, will also guide the number and age of mares to be treated in the individual-based field trials. Modeling will be conducted to guide the duration goals for the research effort to explore the following: (a) the best handling strategy (injection during gathers vs. remote injections); (b) the most optimum treatment plan (treat young mares only vs. a random selection of mares); (c) marking (mark all treated mares vs. mark none); (d) optimum efficacy; (e) strategies to achieve the minimal number and frequency of handlings to minimize the number of horses to be adopted; (f) strategies to minimize costs; (g) optimal monitoring efforts for a given product; and (h) the best mix of strategies (removal only, contraception only, or both) to meet the BLM's stated objectives.

VII. EXPERIMENTAL DESIGN

A herd-specific animal treatment plan would be developed to determine the number, age, and area of mares to be targeted for the contraception, following the recommendations of the Wild Horse and Burro Advisory Board (criteria sent to Lee Delaney, BLM, 29 April 1999). Once the plan has been developed, the population effects of the contraception would be forecasted. The guidelines established from the conceptual modeling should be followed for the targeted number of animals to be contracepted (versus removed), influence upon growth rate, and influence upon age/sex ratios in accordance with the goals for herd management as stated in the herd management plan and any NEPA documents describing the plan prepared by the field office.

A. Individual-Based Study Herds (Small Populations)

Mares in three or four herds were selected in 2002 and 2003 for intensive study. Individuals were recognized from natural markings using a computerized photo ID system called WHIMS (Wild Horse Information Management System, USGS-BRD, Ron Osborne. Final report to BLM 1999). Individual behavior, reproduction, survival, and any health abnormalities will be closely monitored in these individually recognized horses. Horse identification, behavioral, and fecundity studies will be conducted by USGS-BRD biological technicians under supervision of BRD research biologists.

All treated mares were permanently marked on the left side of the neck with a freezebrand that was entered into the WHBIS (Wild Horse and Burro Information System) database. This number will be used to track the animal in the event the horse was ever removed from the range. Two exceptions have been granted to this ruling – the Pryor Mountains and Little Book Cliffs herds – where public objections to the branding occurred, tracking by individual animal markings is assured, the herds are isolated, and animals escaping from fenced boundaries are rare.

Treated mares will be compared to untreated mares (controls) in the same harems. Multivariate models would include age of mare, year, weather, density-dependent relations, and

compensatory responses. If possible, harems with no treated mares will also be observed. Pretreatment data on harem dynamics, population dynamics, and behavior will be gathered prior to contraception, wherever possible. Two years of pretreatment information on any population to be treated will be highly desirable. In those instances where this is not possible, the experimental unit will be treated and untreated mares from the time of post-treatment only.

B. Population-Based Study Herds (Large Populations)

A number of population-based study herds have also been selected for research with less direct observation. More herds are intended to be added to this study. During capture, gather, freezemarking and injection, most of the same information will be gathered on treated mares as is gathered for the individual-based study herds described above. Monitorings will be done by helicopter assessment of herd growth rates and more obvious health-related issues. Mares will be identified only as treated or untreated, and will not be individually recognized.

The marking system proposed for the Population-Based Fertility Control Trials consists of two 3 ½" letters applied to the left hip of each treated mare. The letters will be assigned consecutively in a basic system. The first trial for FY04 would be assigned the letters AA – the second trial AB – the third AC and on down the line. The next FY would begin with the letter B and then assigned consecutively BA, BB, BC etc. The letters will be assigned by NPO as gathers and fertility control are approved. The vaccine is maintained at NPO so the freeze irons will be shipped out with the vaccine. A copy of the data sheet will be maintained at NPO filed consecutively with the freeze letter used stamped at the top of the data sheets. All information pertinent to a particular Population Based Trial will be maintained in this file.

One primary reason for the selection of letters is that they have rarely been used by BLM. Most freeze marks that have been applied (for numerous reasons) have been numbers. It is assumed that all Population-Based Trials in the near future will use the 22-month time release. In the event re-treatment is proposed and approved an X could be added after the original freeze mark. NPO will track all PZP use. Vaccine issued but not used will be shipped back to NPO and stored for future use.

VIII. CRITERIA FOR STUDY HERD SELECTION

Individual-Based Study Herds	***Population-Based Study Herds***
(a) Animal access (gather) must be planned.	(a) Same.
(b) An EA must be prepared and approved on the gather and the contraception.	(b) Same.
(c) The herd should have prior high quality data on herd history, population size, and growth rates.	(c) Same.
(d) The herd manager(s) must be open and supportive to the investigators and must voluntarily offer the herd unit up for the research.	(d) Same.

Individual-Based Study Herds	*Population-Based Study Herds*
(e) It is desirable, but not essential, that prior genetic information be available for the herd. In either case, genetic information must be gathered on the treated and any untreated mares that are released back into the population at the time of the contraceptive treatment.	(e) Same.
(f) The manager(s) must be willing to leave treated mares on the range for the duration of the research, including through post-treatment observations.	(f) Same, except specific mares will not be observed post-treatment. Any study mares that are treated with both PZP and FCA may not be adopted until three years after treatment has occurred.
(g) A site-specific population model prescription will be conducted, identifying the number, age of the animals to be treated, and the projected affects on population rate, as recommended by the BLM Advisory Board, April 1999.	(g) Same.
(h) A foal-sized freeze brand will be placed on the left side of the neck of the treated mares, compatible with WHBIS. Information documenting the agent, adjuvant, and date of injection will be recorded with this identification number.	(h) The marking system proposed for the Population-Based Fertility Control Trials consists of two 3 ½" letters applied to the left hip of each treated mare. The letters will be assigned consecutively in a basic system. The first trial for FY04 would be assigned the letters AA – the second trial AB – the third AC and on down the line. The next FY would begin with the letter B and then assigned consecutively BA, BB, BC etc.
(i) Good access is essential.	(i) Good access is not essential, but aerial surveys must be effective.
(j) The herds should be biologically discrete from any other nearby herds (WH&B Advisory Board, April 1999). If several subpopulations exist, the fertility control can be evaluated if all units are treated with a uniform protocol and the entire area is surveyed, as if it is one unit.	(j) Recommended the same.

IX. INFORMATION TO BE COLLECTED ON THE STUDY HERDS

Population Data	**Population Data**

Individual-Based Study Herds	***Population-Based Study Herds***
(a) Annual estimates of population size.	(a) One census flight, either year three or four following the treatment year, and prior to any subsequent gather. Some herds may be selected for more frequent census flights.
(b) Annual estimates of reproductive rate.	(b) Not feasible.
(c) Annual estimates of foal: adult mare ratios and stallion: adult mare ratios.	(c) refer to IX. (a) above.
(d) Determine age-specific fecundity and survival rates for treated and untreated mares through close ground monitoring of all individually identifiable animals. Determine any effects of contraception on survival or fecundity post-treatment. Determine compensatory fecundity or survival due to contraception.	(d) Determine herd growth rates only through aerial surveys. Determine any effects on population growth rate of a single application of the 22-ms agent, within the 4-year cycle.
Model Prescription of Treatments Prior to Contraception at the Gather	**Model Prescription of Treatments Prior to Contraception at the Gather**
(a) Determine age and location of mares to be treated.	(a) Same.
(b) Estimate of effect of the treatment on viable population size, population goals, reproductive rate, genetics, and forecast population effects of contraception.	(b) Forecast the effects of fertility control treatment on population growth rate and population size only.
Data Recorded During the Initial Gather[3]	**Data Recorded During the Initial Gather[3]**
(a) Administration date, type of agent (1or 2 year PZP) and adjuvant.	(a) Same.
(b) Percent of mares treated and released.	(b) Same.
(c) Pregnancy status of treated and any untreated mares released, determined from a blood, urine or feces sample.	(c) Not feasible.
(d) Treated mares stratified by known ages.	(d) Treated mares stratified by rough age categories determined by aging of mares during the gather handling, either by a veterinarian, contractor, or BLM personnel experienced in aging.

[3] Individuals responsible for vaccine application are asked to fill out reporting forms at the gather specific to the type of field trial. The data collection for the large population trials is minimal and streamlined. Please send copies to NPO Reno and USGS-BRD.

Individual-Based Study Herds	*Population-Based Study Herds*
(e) Mares (and all other released animals) will be individually recognizable by photos taken at gather and entered into WHIMS photo ID system.	(e) Mares will be identified by a hip brand. Untreated mares will carry no such freezemark.
Pregnancy & Date of Foaling Information	**Pregnancy & Date of Foaling Information**
(a) Determine any pregnancy of treated and untreated (control) mares for 2-3 years using noninvasive urine or fecal collections from the individual mares and pregnancy analysis in one or two select herds only. Determine any foaling of treated mares from ground observations. Determine foaling and dates of foaling of untreated mares and treated mares subsequent to treatment through intense ground monitoring of harems. Compare any extended estrus cycling of mares through behavioral estrus and/or urine analysis from a sub-sample of non-pregnant treated and untreated mares in one or two select herds.[4]	(a) Not possible in these herds.
(b) Determine any influence of contraceptive treatment on season of foaling through weekly ground monitoring of all individual mares, both treated and untreated.	(b) Not possible.
(c) Determine fertility of foals, as adults, that were born to contracepted mares or born later to these mares following cessation of contraceptive treatment.	(c) Not possible.

[4] This kind of information gathering likely will not be feasible with free-ranging herds. Individual mares would need to be found and sampling of feces or urine made every two to three days, year round. Studies will need to be conducted on mares in a captive setting to determine estrus and clinical complications. Complete information on estrus cycling cannot be obtained from free-ranging animals.

Individual-Based Study Herds	*Population-Based Study Herds*
Monitor Any Complications Due to Contraception	**Monitor Any Complications Due to Contraception**
(a) Record any granulomas, abscesses, lameness, behavioral depression, tremors, or obvious neurological problems in the field, post-release. Closely monitor animals in corrals for 12 hours post-injection, prior to their release back into the wild. Document how these complications were searched for: observation techniques, number of hours of observation, animals observed, and distance to the animals.	(a) Not possible (except for close monitoring in the corrals prior to the release).
(b) Record body condition (Henneke System) of treated and untreated mares within the same harem using the Henneke System.	(b) Not possible.
Behavioral Responses to Contraception	**Behavioral Responses to Contraception**
(a) Determine any effects of contraception on the vigor of any foals born to treated mares following their return to fertility.	(a) Not possible.
(b) Document any effect of contraception on mare retention in the harem; isolation or disassociation of treated mares; disinterest either by mares or stallions in normal harem dynamics; and spatial locations of treated vs. untreated mares within the harem grouping.	(b) Not possible.
(c) Determine whether treated mares interchange harems more frequently than untreated mares.	(c) Not possible.
(d) If estrus is extended for treated mares,[5] determine whether there is any greater energy expenditure of mares or stallions.	(d) Not possible.
(e) Compare harem behavior of treated and untreated mares, including dominance relations of the mares, stallion tending of mares, mare behavior, and mare harem interchange rates.	(e) Not possible.

[5] See Section XV "Related Studies in Captivity."

Individual-Based Study Herds	*Population-Based Study Herds*
Pretreatment Information	**Pretreatment Information**
(a) In every herd where feasible, two years or more of pretreatment information on foaling dates, fecundity, survival rates, and behavior should be gathered. Two of the herds have several years of pretreatment information (Pryor Mountains and Little Book Cliffs). Adequate controls to the treatments are absolutely necessary for statistical power to evaluate the effects of the contraception.	(a) Pretreatment information should be available from previous BLM aerial surveys and gathers on population size, age structure, sex ratio, foaling season and population growth rate.

X. RELATED STUDIES IN CAPTIVITY

Tests to replace the less desirable FCA with Modified Freund's Adjuvant (MFA), or another adjuvant, are best conducted in a captive setting. Mares need not always be bred or fertilized. Research for some agent mixtures need only focus on titer levels (presumed efficacy) and time that titer levels are raised (presumed duration). Where the relationship between titer levels and contraception is well established, only the titer levels need to be monitored. By conducting studies on horses in a captive setting, all held under the same conditions, environmental variation and sampling variation can be eliminated or greatly reduced. Sample sizes can be smaller and the number of study animals and study costs can be reduced.

The intensity of sampling required to monitor estrus cycles in mares is so high it will not be possible to collect the required samples from free-ranging wild mares. The ideal solution may be to tie this work to one of the studies of agents in a captive setting. Wild horses differ from domestic horses in a number of subtle and significant ways (e.g. wild horses' annual estrus cycle would likely occur over a narrower period than domestic horses). These differences may be purely environmental or inherited. Wild horses in pens could be used where the potentially unique physiology, body condition, and adaptations of wild horses might influence the research findings. Domestic horses, however, are preferred for any invasive research.

XI. INTEGRATION WITH AERIAL SURVEY AND GENETICS RESEARCH

Four aerial survey techniques will be tested on the study populations. Truth is defined as either the known number of animals in the study herds or a known number of horses removed. The best technique(s), in terms of estimating the true number of wild horses, will be selected for broader application to other herds. Animal numbers in the study herds will be determined from photo ID system or known removals.

Herd genetic diversity (heterozygosity) and allelic diversity will be sampled at the time of gather and treatment, as well as five and ten years subsequent. The effects of contraceptive applications on genetic heterozygosity will be determined, for the Individual-Based study herds.

Greater details on the integrated research programs are available in the Strategic Research Plan for the Wild Horse and Burro Program.

XII. LITERATURE CITED

Gross, J.E. 2000. A dynamic simulation model for evaluating effects of removal and contraception on genetic variation and demography. Biological Conservation 96:319-330.

APPENDICES

APPENDIX 1: Criteria Identifying Ideal Contraceptive Agent

The following criteria have previously been identified by federal agencies (NPS and BLM) as requirements for an ideal contraceptive agent. Below each criterion is a list of published papers, specific to horses, which address the merit of PZP vaccine in each category. This vaccine has been in existence for more than 35 years and there also exists many more references specific for deer, elk, zoo animals, African elephants and non-human primates.

1. The agent should be at least 90% effective.
Liu et al. 1989. J. Reprod. Fert. 89:19-29
Kirkpatrick et al. 1990. Wildl. Soc. Bull. 18:326-330.
Kirkpatrick et. al. 1992. J. Reprod. Fert. 94:437-444.
Kirkpatrick et al. 1995. Bio. Reprod. Monograph Series 1: Equine Reproduction VI: 411-418.
Turner et al. 1996. J. Reprod. Fert. 107:31-35
Turner et al. 2001. J. Wildl. Manage. 65:235-241.
Turner and Kirkpatrick 2002. Reproduction (Suppl. 60): in press

2. The agent should be capable of administration by remote delivery.
Kirkpatrick et al. 1990. Wildl. Soc. Bull. 18:326-330.
Kirkpatrick et al. 1991. J. Reprod. Fert. (Suppl. 44): 321-325.
Turner et. al. 1992. J. Wildl. Manage. 56:154-157.
Kirkpatrick et al. 1995. Bio. Reprod. Monograph Series 1, Equine Reprod. VI: 411-418.
Turner et al. 1996. J. Reprod. Fert. 107:31-35.

3. The agent should be reversible.
Liu et al. 1989. J. Reprod. Fert. 89:19-29
Kirkpatrick et al. 1991. J. Reprod. Fert. (Suppl.44): 321-325.
Kirkpatrick et al. 1992. J. Reprod. Fert. 94:437-444.
Kirkpatrick et al. 1995. Bio. Reprod. Monogr. Series 1: Equine Reproduction VI: 411-418.
Turner et al. 1996. J. Reprod. Fert. 107:31-35
Turner et al. 1997. J. Wildl. Manage. 61:873-880.
Kirkpatrick and Turner, 2002. Reproduction (Suppl. 60): in press

4. The agent should be safe for pregnant animals.
Kirkpatrick et al. Wildl. Soc. Bull. 18:326-330.
Kirkpatrick et al. 1992. J. Reprod. Fert. 94:437-444.
Kirkpatrick et al. 1995. Bio. Reprod. Monograph Series 1, Equine Reprod. VI: 411-418
Kirkpatrick and Turner 2002. Reproduction (Suppl. 60): in press.

5. The agent should not pass through the natural food chain.
The PZP vaccine is simply glycoprotein in nature (Dunbar et al. 1980. Biochemistry 19:356-365). Proteins are not absorbed whole because they are digested. Even in cases where the digestion is not complete, the digestion products are di- or oligopeptides, which are not biologically active.

"It has been demonstrated that proteins are not completely hydrolyzed to amino acids before absorption, but that a certain fraction is absorbed as di- or oligopeptides, which are hydrolyzed inside the mucosal cell." (Newey and Smith 1960. J. Physiol. 152:367.). Any freshman level biochemistry or physiology text will provide basic information about the failure of proteins to be absorbed in biologically active forms.

6. The agent should be inexpensive.
Under the INAD authorization the vaccine must be made available for no more than the cost of production, which is currently $20/dose (65 µg).

7. There should be no debilitating side effects on the health of the horses.
Kirkpatrick et al. 1992. J. Reprod. Fert. 94:437-444.
Kirkpatrick et al. 1995. Bio Reprod. Monograph Series 1, Equine Reproduction VI: 411-418.

8. The agent should not influence the social behavior of the horses.
Kirkpatrick et al. 1990. Wildl. Soc. Bull. 18:326-330.
Kirkpatrick et al. 1992 J. Reprod. Fert. 94:437-444.
Powell 1999. J. Appl. An. Welfare Sci. 2:321-335.

9. The treatment must be easy to store, handle, and apply under field conditions and not represent a significant risk for BLM employees or their contractors.
The PZP vaccine must be stored frozen. It can be lyophilized but it loses biological activity and the cost begins to increase because larger volumes are necessary. It can be thawed out prior to use and re-frozen. It is usually mixed with the adjuvant just before use, but also can be pre-mixed. This tends, however, to result in significant loss of PZP and an overall increase in cost.

The vaccine has been used in this form for 14 years on Assateague Island National Seashore, under very poor field conditions (as opposed to a sheltered area next to a chute) without problems. Risks are limited to users as it cannot be taken orally or absorbed through the skin. After 14 years there are at least 12-15 (at least 50% are women) people who have administered the vaccine annually, in frequent darting activities, and there have been no reported problems of any kind. In addition, approximately 100 zoo veterinarians administer the vaccine on a regular basis and have reported no problems.

APPENDIX 2: QUALITY CONTROL OF PORCINE ZONA PELLUCIDA CONTRACEPTIVE VACCINE

The Science and Conservation Center
ZooMontana
2100 South Shiloh Road
Billings, MT 59106

Research Year 2002-2003:

Porcine zona pellucida (PZP) contraceptive vaccine is prepared at The Science and Conservation Center (SCC) according to modified techniques of Dunbar et al. (Biochemistry 80: 356-365, 1980). Each batch of PZP, which constitutes about 200 horse doses of 100 ug PZP, is subjected to a series of quality control tests. These tests include:

1. **Quantitative analysis:** The actual protein content of each batch is determined by a whole protein assay. The assay is the Bio-Rad DC (detergent compatible) protein assay, which is a modified method of Lowry, et al. (J. Biol. Chem. 193:265, 1951).

2. **Qualitative analysis:** Each batch of PZP is subjected to polyacrylamide gel electrophoresis (PAGE), which separates and illustrates all proteins included in the PZP. All PZP made by any lab contains some soluble proteins that cannot be removed completely, such as albumins and perhaps some collagins, and the gel identifies the presence of these proteins, as well as the ZP proteins. Gels are dried and kept on file at the SCC.

3. **Viral testing:** Samples of PZP are sent to the USDA laboratory at Ames, Iowa for testing for pathogenic hog viruses, including PRRS, pseudorabies, encephalomyocarditis, hemagglutinating encephalomyelitis, transmissible gastroenteritis, parvovirus, swine influenza, rotovarus, adenovirus and enterovirus. Most of these are hog specific viruses.

4. **Bacterial screen:** All vaccine is plated on blood agar plates to determine if any bacteria, gram positive pathogens, or others are present.

The results of quality control for each batch are stored at the SCC. The purpose is to be able to counter any claims that the vaccine is harming the animals or causing diseases or toxicity. To date, SCC produced PZP is the only PZP vaccine that is subjected to this entire quality control process.

APPENDIX 3: 2002 PROTOCOL for the TREATMENT of WILD HORSES on the PRYOR MOUNTAIN WILD HORSE RANGE with a PORCINE ZONA PELLUCIDA CONTRACEPTIVE VACCINE

1. PURPOSE

This is a research field trial designed to delay pregnancy in young wild horse mares within the Pryor Mountain Wild Horse Range (PMWHR) through the use of a native porcine zona pellucida (PZP) contraceptive vaccine delivered remotely, under field conditions. The vaccine would induce one year of infertility, allowing the mares to mature in a healthier condition, before becoming pregnant and producing and supporting a foal. In 2002, 22 selected yearling and two year old mares would be treated remotely with the vaccine. The method of delivery would be with 1.0cc Pneu-Darts® and delivery would be by Dan-Inject or PneuDart capture gun.

II. PARTICIPANTS

Project Manager:	Linda Coates-Markle, State Wild Horse and Burro Specialist, BiFO, BLM
Horse Identification:	Field-trained and experienced BLM Seasonal employees.
Vaccine Preparation:	Robin Lyda, The Science and Conservation Center, ZooMontana, 2100 South Shiloh Road, Billings, MT 59106
Designated Darters:	Jay F. Kirkpatrick; Science and Conservation Center; Kim Frank, The Science and Conservation Center
Project Veterinarian:	Lyle Bischoff, DVM Powell Veterinary Service 522 S. Division, Powell, WY 82435

III. PROCEDURES

A. Vaccine preparation and shipment: Vaccine would be prepared under the supervision of Robin Lyda, Science and Conservation Center (SCC), Billings, MT and transported to the field site in Montana on dry ice, under Food and Drug Administration authority (Investigational New Animal Drug exemption No.8857 G0002 & 0003). FDA form "Notice of Drug Shipment" would be completed for each shipment of the PZP vaccine and filed in the offices of the Science and Conservation Center at Zoo Montana, Billings, MT. On the PMWHR, vaccine would be stored frozen in the field.

B. Selection of subject animal: Animals to be treated have all been previously identified by BLM personnel and are identified in EA # MT-010-02-22. The number and identity of animals would be selected on the basis of predetermined animal welfare goals. All animals selected for treatment would be female and at least one year old. If the identification of any

horse is questionable, that horse would not be darted, and the ultimate decision rests jointly with the darter and BLM horse identifier.

C. Delivery of contraceptive vaccine: Delivery of vaccine would be by means of 1.0 cc Pneu-Darts®, with 1.5" barbless needles. 0.5 cc of the PZP vaccine (in phosphate buffered saline, or PBS) would be emulsified with 0.5 cc of adjuvant and loaded into darts at the time a decision has been made to dart a specific mare. Animals which have never been treated would be treated with PZP + Freund's Complete (or Freund's Modified) adjuvant, while animals which have been previously treated would be given PZP + Freund's Incomplete adjuvant. Only designated darters would mix the vaccine/adjuvant and prepare the emulsion. Vaccine-adjuvant emulsion would be loaded into darts at the darting site and delivered by means of a capture gun.

As necessary, the BLM Wild Horse and Burro Specialist would certify those personnel authorized to conduct feral horse darting operations in the PMWHR. At a minimum, authorization would be restricted to those individuals who have documented and successful experience darting wildlife under field conditions, and are specifically authorized to dart PMWHR horses by the Wild Horse and Burro Specialist. In addition, at least one of the designated personnel in any darting operation must have successfully completed the National Park Service (NPS) or a comparable wildlife immobilization training course.

The decision to dart a horse would ultimately rest with the darter. The accessibility of the horse at a particular point in time and location would trigger the decision-making process. Safety, for both humans and the horse is the foremost consideration in deciding to dart a mare. The Dan Inject gun would not be used at ranges in excess of 30 meters and no attempt would be taken when other persons are within a 30 m radius of the target animal. If a darting attempt is not taken, the gun would be unloaded and the dart stored in a poly-foam container. If a loaded dart is not used within two hours of the time of loading, the contents would be transferred to a new dart before attempting another horse. If the dart is not used before the end of the day, it would be stored under refrigeration and the contents transferred to another dart the next day. Refrigerated darts would not be used in the field.

Use of the Pneu-Dart® capture gun for dart delivery is often preferred in the field. Safety is again the foremost consideration. Only low velocity (brown) or medium velocity (green) charges would be used in this project. The gun would remain unloaded until the horse has been selected for a darting attempt. No attempts would be taken at ranges greater than 50 yards. No attempts would be taken when other persons are within a 90N angle defined by a line from the darter to the horse. Only hip or gluteal muscle regions of the horse are acceptable targets. No attempts would be taken in high wind or when the horse is standing at an angle where the dart could miss the hip/gluteal region and hit the rib cage. The ideal angle is when the dart would strike the skin of the horse at a perfect 90N angle. If a horse moves out of firing range after the gun is loaded and it is apparent that another attempt would not be immediately possible, the gun would be unloaded (both cartridge and dart) and stored. Immediately after firing, the empty cartridge would be ejected, and the dart port opened. Every day the capture gun would be used in the field, early morning practice would be required in order to assure that the gun is properly sighted.

It is suggested that no more than two people be present at the time of a darting. The second person should be responsible for locating fired darts. The second person should also be responsible for identifying the horse and keeping onlookers at a safe distance. The safe distance would be determined by the conditions at the time of darting and specifics of the animal involved and darting location.

Fatigue is a concern for darters. Proper treatment of animals requires a clear mind and decisions about veterinary care require careful thought and appropriate responses. Fatigue would not be uncommon because of the hours and habitat associated with horse tracking. It would be the darter's responsibility to determine when work would cease because of fatigue among team members. Weather can also be an important factor and high winds would be a legitimate cause for stopping the operation. The final decision rests with the darter.

To the extent possible, all darting would be carried out in a discrete manner. However, if darting is to be done within view of non-participants or members of the public, an explanation of the nature of the project would be carried out either immediately before or after the darting. Copies of a one page explanation of the project shall be carried by the participants and given to any non-participants at every opportunity.

D. Recovery of darts: Attempts would be made to recover all darts. If possible, all darts which are discharged and drop from the horse at the darting site would be recovered before another darting occurs. In exceptional situations, with the decision resting with the darter, the site of a lost dart may be noted and marked, and recovery efforts made at a later time. All discharged darts would be examined after recovery in order to determine if the charge fired and the plunger fully expelled the vaccine.

E. Record keeping: BLM and SCC personnel would maintain records for the identification of all horses to be darted or used for control purposes. These records would be used to meet FDA regulations for use of the vaccine under the existing INAD. Each horse darted would be permanently identified by the 4-digit BLM identification number. For each horse darted, the following information would be recorded at the time of darting:

1. Identification of darter
2. Date of inoculation
3. Size of PZP dose
4. Type of adjuvant
5. Type of dart/delivery system
6. Precise site of inoculation (right or left side of hip)

Additionally, other observations regarding estrous behavior, presence of abscesses or other concerns, and any other pertinent information collected by researchers or the Wild Horse and Burro Specialist would be maintained by the BLM.

At a minimum, foal counts and birth records shall be carried out annually by BLM personnel. These data shall be recorded by BLM field technicians and transferred to both permanent BLM and SCC records. Other data on mare body condition, fitness and behavior shall be

collected under the guidance and research protocol set by the BLM National Wild Horse Fertility Control Field Trial program.

F. Veterinary Emergencies: Personnel conducting darting operations shall be equipped with a two-way radio or cell phone providing a communications link with the Wild Horse and Burro Specialist. In the event of a veterinary emergency, darting personnel would immediately contact the Wild Horse Specialist, providing all available information concerning the nature and location of the incident. As appropriate, the Wild Horse Specialist would contact the Project Veterinarian for advice and/or assistance.

In the event that a dart strikes a bone or imbeds in soft tissue and does not dislodge, the darter would follow the affected horse until the dart falls out or the horse can no longer be found. The darter would be responsible for daily observation of the horse until the situation is resolved. Possible reasons for a decision to immobilize a horse may include a suspected broken leg, severe lacerations, a dart which has lodged in a bone for more than two weeks, or a severe infection resulting from a dart which is lodged in a bone or the abdominal cavity. The former are all considered to be rare events in normal field darting practices. Other injuries which may occur as a direct result of the darting process, such as severe lacerations and infections, may also require the capture and/or immobilization of the horse for evaluation and treatment. Any decision to capture or immobilize would be made in consultation with the Project Veterinarian. Whenever possible, corralling techniques would be used to capture and contain injured horses. If, in consultation with the Project Veterinarian, the use of immobilizing drugs is deemed necessary and appropriate, such agents would be administered exclusively by the Project Veterinarian or by a member of the darting team under the *direct* supervision of the Project Veterinarian.

All injuries would be treated as per the recommendations of the Project Veterinarian, in consultation with the Wild Horse and Burro Specialist. In the event of a broken leg, or other severe injury, where the Project Veterinarian considers the prognosis for full recovery unlikely, the affected horse would be humanely euthanized, after consultation with the Billings Field Manager and the Wild Horse and Burro Specialist.

G. Blood samples/recovery of ovaries: An attempt to recover blood samples for antibody analysis and to recover ovaries for determination of ovarian effects shall be carried out opportunistically. In the unlikely event that a female horse inhabiting the PMWHR must be euthanized for humane reasons, a blood sample would be immediately collected in a red top10 cc tube. The sample would be sent to the Project Veterinarian where the serum would be harvested and stored frozen. If at all possible, at least one and preferably both, ovaries would be excised and placed in 10% buffered formalin, for histological examination.

H. Media relations: All requests by the media (verbal, written or electronic), must ultimately pass through the Wild Horse and Burro Specialist, BiFO, Billings, MT or their designate, and the decision to release information related to the project shall rest with the BLM. Efforts would be made to inform media and other interested public as to the status of darting efforts on the PMWHR on a daily basis during planned activity.

I. Public Relations: A public communications plan would be prepared soon after the release of the EA addressing the proposed action. Prior to the start of darting activity, the BLM shall distribute to all law enforcement agencies with jurisdiction on the PMWHR, a notice that darting would commence on a particular date and end on a particular date, and that darters may be witnessed by members of the public darting horses with a capture gun. This information would minimize panic calls from a concerned public and provide law enforcement with an opportunity to explain the circumstances and direct the public to BiFO for further details of the operation.

J. Reporting: An annual report would be prepared by the BLM Wild Horse and Burro Specialist documenting contraceptive program activities, impacts on the PMWHR herd and program status, successes and/or concerns. At the completion of the research field trial, all results would be analyzed and reported pertaining to guidance and research protocol set by the BLM National Wild Horse Fertility Control Field Trial program.

APPENDIX 4: Draft Criteria for Immunocontraceptive Use in Wild Horse Herds

Prepared for: Lee Delaney and the Wild Horse & Burro Advisory Board
Prepared by: Dr. Allen Rutberg, HSUS, BLM WH&B Advisory Board Member
Date: April 29, 1999
Accepted by BLM on August 16, 1999 for implementation.

Issue Summary: The Wild Horse and Burro (WH&B) adoption program has been the BLM's only acceptable outlet for removing excess animals from public lands. This program has not been able to find acceptable homes for all the animals that have been removed from the range to reach Appropriate Management Levels.

Agency Position: The BLM's WH&B program has been faced with the task of achieving AML in wild horse herds in a specified time frame. With an average recruitment rate of 18-24% in healthy horse herds, the time frame has become a moving target which is difficult to achieve with the adoption program as the only outlet for excess horses. The immunocontraceptive vaccine (PZP) has been used strictly as a research tool on wild mares. The vaccine now should be tried as a management tool.

Background: The BLM's WH&B program has been investigating contraceptive methods since 1985. The National Research Council funded research using Nevada and Oregon wild horse herds. The first research dealt with steroid implants in the necks of mares mainly between the ages of 3 and 12 years with some implants done in older mares. All of the treated horses were radio collared along with a control group. The implants were successful in controlling fertility for a 28 month period in most of the mares. Dominant stallions were also gelded in an attempt to limit reproduction. Both studies were completed in 1989. However, there were serious problems with the radio collars, the invasiveness of the implant procedure, and long-term effects of the steroid implants, so additional research was not pursued.

BLM-sponsored research on the PZP immunocontraceptive vaccine has been carried out since 1992. At the present time the vaccine is only effective for 1 year. The current window of application is October through February for maximum effectiveness. A 2-year vaccine may be ready for field use in the fall of 1999. However, the BLM believes the PZP vaccine is ready to try now as a management tool.

Immunocontraception needs to be dealt with in HMAs on a case-by-case basis to address specific problems and should not be used wholesale on all herds. HMAs proposed for fertility control application should meet the following criteria:

1. The need to apply the vaccine to control wild horse population size in a given HMA or complex of HMAs must be identified and documented. Documentation should include habitat monitoring data and reliable information on wild horse population size and reproductive rates.

2. Fertility control should be applied to biologically discrete populations, covering more than one HMA if there is significant exchange of horses between HMAs.

3. A site-specific population model should be developed to determine the number of animals to be targeted for contraception to achieve specified objectives, and to forecast the population effects of contraception.

4. Contraception must not be used in a manner that threatens the health of individual animals or the long-term viability of any herd.

5. Site-specific minimum viable population size should be identified based on:
   a. Number of animals
   b. Reproduction rates
   c. Age/sex ratios
   d. Environmental and range conditions and stability

6. Fertility control should only be applied on HMAs where AMLs have been set.

7. The BLM must complete necessary NEPA documentation prior to utilizing fertility control. Preparation of this documentation must be timely enough to provide adequate opportunities for public review.

8. A protocol for administering the vaccine must be developed, including rules for selecting specific animals for treatment. These rules should follow the "minimum feasible level of management" language of The Act. Ongoing discussions among BLM staff, researchers, and the public should be conducted to allow these protocols to evolve with growing understanding of horse behavior, genetics, and the impacts of fertility control.

9. Gathers at which fertility control would be used must be coordinated through the National Program Office.

Public Interest: The public is very interested in all aspects of the WH&B Program. Many feel we should not interfere with the animals on the range; there are also concerns' with long-term effects of fertility control on horse behavior and herd viability. With adequate monitoring and guidelines, however, this could be a broadly acceptable way of controlling horse herd growth rates.